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Structural Basis of Gating by the Outer Membrane Transporter FecA

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Scientists working at the NSLS and Argonne National Laboratory's Advanced Photon Source (APS) have determined the crystallographic structure of the outer membrane receptor FecA from Escherichia coli with and without ferric citrate – an essential nutrient of bacteria – at 2.5 and 2.0 angstrom resolution. This study establishes the structural basis of gating for receptors dependent on the cytoplasmic membrane protein TonB.

Despite its relative abundance in Earth's crust, iron is biologically unavailable in the atmosphere. So, in response to iron deficiency, most microbes secrete organic chelators – compounds having a central metallic ion attached to two or more nonmetallic atoms – called siderophores, which are designed to sequester ferric iron.

Bacteria survive by using highly selective mechanisms designed to actively pump iron across the cell envelope. These mechanisms, responsive to both the internal and external iron concentration, control the transcription of genes involved in iron uptake. The ferric citrate uptake (*fec*) genes are responsible for the transport of ferric citrate from the external medium into the cell. Embedded within the outer membrane is FecA, a receptor that binds and transports ferric citrate, and is required to initiate transcription of the *fec* uptake genes.

We have determined the architecture of FecA from *Escherichia coli* and its gating mechanism by x-ray crystallography. Using mixed detergent-protein micelles, FecA crystals with and without ferric citrate were grown. Data was collected to 2.0 Å from unliganded FecA at beamline X12C of Brookhaven National Laboratory's National Synchrotron Light Source; and to 2.5 Å from liganded FecA at the Argonne National Laboratory's Advanced Photon Source.

Johann Deisenhofer, professor of biochemistry and investigator in the Howard Hughes Medical Institute at the University of Texas Southwestern Medical Center in Dallas, conducted the study on the outer membrane transporter FecA with lead author and postdoctoral researcher Andrew Ferguson (not shown in the picture). Deisenhofer received the 1988 Nobel Prize in Chemistry.

FecA is composed of three domains (Figure 1): (i) a 22-stranded antiparallel β-barrel embedded within the outer membrane, with long extracellular loops and short periplasmic turns; (ii) a "plug," consisting of a mixed four-stranded β-sheet with short interspersed helices, and extracellular and periplasmic pockets, located above and below the plug; (iii) a disordered third domain, the NH_2-terminal extension, which resides entirely within the periplasm and is required for the initiation of transcription.

Ferric citrate binding affects the conformation of the barrel and the plug domain of FecA by causing a dramatic change in the spatial arrangement and conformations of primarily the seventh and eighth extracellular loops, as shown in Figure 2.

From our structural observations, we propose the following transport mechanism.

Stage 1: Ferric citrate is adsorbed from the medium primarily by aromatic residues found within the upper portion of the external pocket of FecA.

Stage 2: Ferric citrate is trans-

ferred to its high-affinity binding site, causing an allosteric transition within the plug that signals the occupancy of FecA in the periplasm.

Stage 3: Multiple extracellular loops of the barrel change their relative conformation and position, thereby closing the external pocket of the barrel.

Stage 4: Transitions that modify the conformation of the plug domain and/or barrel are needed for transport to occur, and are mediated by physical interactions between FecA and the cytoplasmic membrane protein TonB.

Our findings clarify the current understanding of energy-dependent siderophore uptake across the bacterial outer membrane, and establish the structural basis of gating for TonB-dependent receptors. Further genetic and biophysical studies are needed to establish the molecular basis of energy-dependent transport.

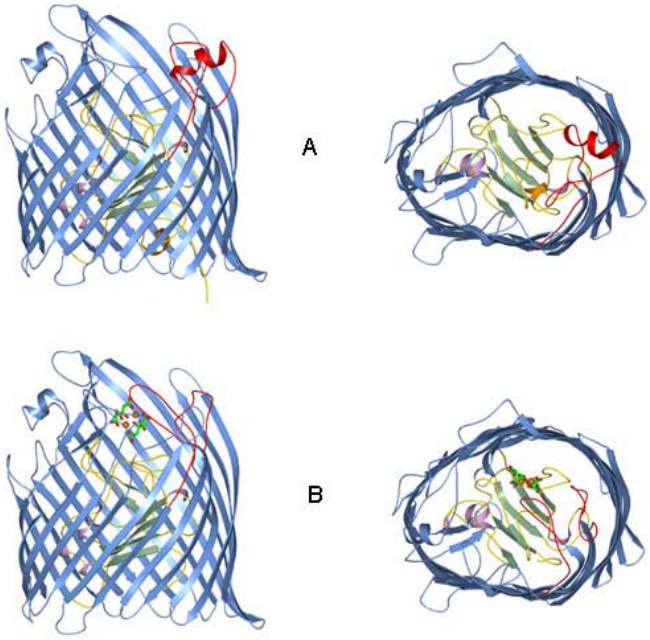

Figure 1: *Crystallographic structure of FecA, (A) unliganded, and (B) liganded. The 22-stranded β barrel is shown in blue. The mixed four-stranded β sheet of the plug domain is shown in green. The switch helix, located in the periplasmic pocket of FecA, is colored orange and is only observed in the unliganded conformation.*

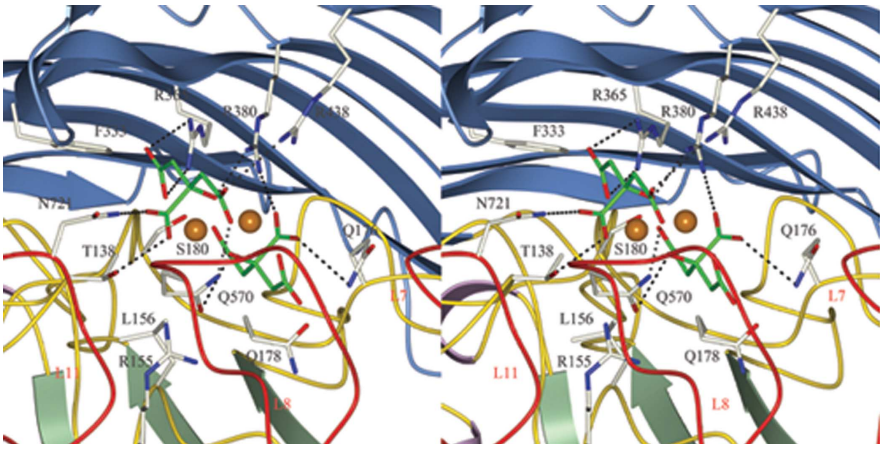

Figure 2: *Stereoview of the ferric citrate-binding site. All side chains within 3.5 angstrom of dinuclear ferric dicitrate are shown with carbon atoms in white, nitrogen atoms in blue, and oxygen atoms in red. The strands and extracellular loops of the barrel are shown in blue; the strands forming the plug domain are in green, and loops are in yellow. The dinuclear ferric citrate molecule is represented with oxygen atoms in red, carbon atoms in green, and ferric ions in orange.*